Plasma nitric oxide metabolite levels increase during successive exercise stress testing – A link to delayed ischemic preconditioning?

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D Zdrenghea, G Bódizs, MC Ober, M Ilea. Plasma nitric oxide metabolite levels increase during successive exercise stress testing – A link to delayed ischemic preconditioning? Exp Clin Cardiol 2003;8(1):26-28.

BACKGROUND: Animal studies have shown that nitric oxide is involved in delayed ischemic preconditioning.
OBJECTIVES: To determine whether plasma nitrates and nitrites (NO_x^-, as measure of nitric oxide) are modified by two consecutive effort tests and whether these changes translate into clinical improvement
METHODS: Twenty-two patients with ischemic heart disease each performed two effort tests at 24-h intervals. Plasma NOx^- level was determined and compared before and after both stress tests. Peak effort, double product at peak effort and maximal ST segment depression were considered clinical endpoints and were compared between the two tests.
RESULTS: Plasma NO_x^- increased slightly after the first exercise test compared with pretest value (17.05±1.6 µmol/mL versus 15.38±1.4 µmol/mL). In turn, after the second test there was a significant rise in NO_x^- level (23.65±2.2 µmol/mL versus 15.10±1.3 µmol/mL, $P<0.03$). The pretest values were almost identical between the two tests. Peak effort and double product at peak effort remained unchanged between the two tests. Although ischemic stress was the same, ST depression was significantly lower ($P<0.01$) for the second test (0.85±0.06 mm versus 1.73±0.16 mm).
CONCLUSION: Our study shows an increased plasma NO_x^- level after the second of two consecutive exercise stress tests at 24-h intervals, along with a decrease of electrocardiographic consequences of approximately the same ischemic stress. These findings are consistent with experimental data in animals, which point to nitric oxide as a trigger and effector of ischemic preconditioning.

Key Words: *Delayed preconditioning; Exercise; Nitrate; Nitric oxide; Nitrite*

Delayed ischemic preconditioning is generally considered the mechanism of the favourable effect of a previous angina on outcome in acute myocardial infarction patients (1-3). It could also be the cause of decreased maximal ST depression under a similar ischemic stress during successive exercise stress testing (4,5) or of the increase in myocardial workload at which similar effects (eg, the same ST depression or angina) occur.

The mechanism of delayed preconditioning seems to involve nitric oxide (NO) as both a trigger and a mediator of the phenomenon (6-8). It was shown that a first ischemic episode increases NO production via constitutive endothelial nitric oxide synthase (eNOS). NO is one of the triggers for transcription of inducible nitric oxide synthase (iNOS) resulting in greater NO production during a second ischemic episode (24 h later), which mediates the protective effect.

Such an increase in NO production during the second ischemic episode was not shown in humans until now.

The purpose of our study was to investigate NO production before and after two consecutive exercise stress tests at 24-h intervals.

METHODS

The study involved 22 patients with ischemic heart disease, which was confirmed by coronary angiography. Thirteen patients presented with stable effort angina and nine patients with old myocardial infarction. Of these, 18 patients were males and four were females, aged 41 to 74 years (mean 56.2±1.6 years). Patients with resting electrocardiogram (ECG) changes that would make exercise ECG difficult to interpret (ie, conduction disturbances, hypertrophy), were excluded.

For all patients, current medications including anti-ischemic drugs were maintained unchanged during the study, but nitrates were excluded a week before.

Exercise protocol: All patients performed two maximal, symptom-limited exercise tests (ETs) on the cycloergometer at 24-h intervals (ET_1 and ET_2), under supervision of the same experienced physician. Both ET_1 and ET_2 were performed in the morning, according to classical protocols (9,10), in increments of 25 W and 2.5 min duration. One bipolar lead (V_5-V_5R) was continuously monitored during the tests and was recorded during the last minute of every step and at peak exercise. During the recovery phase, a 12-lead ECG was recorded at 1 min, and every 2 min after, until 9 min or until angina and ECG changes disappeared. ECG was automatically analyzed for ST changes at 0.06 s from the J point. Maximal ST depression was considered in lead V_5-V_5R during exercise or in the lead with maximum ST depression if that appeared only in the recovery phase. Blood pressure was manually measured by the same pattern as the ECG recording. A test was considered positive if a maximum ST depression of at least 0.1 mV occurred.

The positivity of ET_1 represented the including criterion. ET_2 was performed after 24 h in similar conditions.

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TABLE 1
Hemodynamic, workload and medication data

	Pre-ET_1	Pre-ET_2	P*	Peak ET_1	Peak ET_2	P**
sBP (mmHg)	131±3.5	136±3.7	NS	170±6.1	173±5.7	NS
dBP (mmHg)	84±1.9	87±1.8	NS	96±2.7	101±2.4	NS
mBP (mmHg)	100±2.3	104±2.1	NS	125±3.4	127±2.8	NS
HR (min^{-1})	72±3.2	67±2.5	0.01	109±6.0	103±5.2	NS
DP (mmHg/min)	9474±503	9184±498	NS	21224±2344	22300±2400	NS
Workload (W)	–	–	–	79.3±7.1	80.3±7.4	NS

*Medications expressed as number of patients (percentage): acetylsalicylic acid 22 (100%), beta-blocker 22 (100%), angiotensin-converting enzyme inhibitor 20 (91%), calcium blocker 3 (14%), nitrate 0 (0%). *Comparing pre-ET_1 and pre-ET_2 values. ** Comparing peak ET_1 and peak ET_2 values. dBP Diastolic blood pressure; DP Double product; ET_1 First exercise test; ET_2 Second exercise test; HR Heart rate; mBP Mean blood pressure; sBP Systolic blood pressure*

For both ET_1 and ET_2, the following parameters were analyzed: peak effort (as a measure of maximum systemic oxygen update [VO_{2max}]), heart rate, blood pressure and double product (DP) at peak effort (as a measure of maximum myocardial oxygen demand [peak MVO_2]) and maximal ST depression.

Nitrates/nitrites determination: It has been shown that plasma nitrates or sum of nitrates/nitrites are measures of endogenous NO production (11). Blood samples were obtained for quantitation of nitrates/nitrites before and after each exercise stress test. Post-test samples were obtained during the recovery phase. In order to determine the level of nitrate/nitrite (NO_x^-), plasma was incubated with reduced nicotinamide adenine dinucleotide phosphate (NADPH) and NADPH-dependent nitrate reductase to convert nitrates to nitrites. Afterwards, the nitrites level was determined by the Griess method (11), using a commercially available analysis kit (Griess Reagent System, Promega Corp, USA) and expressed as µmol/mL. The investigators who performed ETs and NO_x^- determinations were blinded to the other investigators' results.

Statistical analysis: Data were compared before and after every ET or between the two ETs (as necessary) using Student's paired *t* test. Data are expressed as mean ± SE. $P \leq 0.05$ was considered statistically significant.

RESULTS

The hemodynamic, workload and medication data on the two tests are presented in Table 1. The analysis of the peak effort as a measure of VO_{2max} revealed no significant difference between ET_1 and ET_2. The mean values of the DP at peak effort, as a measure of peak MVO_2, were also very close during ET_1 and ET_2. Blood pressure and heart rate at peak exercise were identical for both tests (Table 1).

In turn, maximal ST depression (Table 2) during ET_2 was significantly less than maximal ST depression during ET_1, $P<0.01$. In five patients (22.7%), the ET_2 became negative (ST depression less than 1 mm).

The plasma NO_x^- level immediately after ET_1 was only slightly higher than rest value before ET_1. After 24 h, before ET_2, the resting NO_x^- level was about the same as before ET_1. In turn, after ET_2, the plasma NO_x^- level was significantly ($P<0.03$) higher than both values before ET_1 and ET_2 (Table 2).

TABLE 2
Plamsa nitrate/nitrite (NO_x^-) and ST depression results

	ET_1		ET_2		P*
	Pre-ET	Post-ET	Pre-ET	Post-ET	NS
NO_x^- level (µmol/mL)	15.38±1.4	17.05±1.6	15.10±1.3	23.65±2.2**	
ST depression (mm)	1.73±0.16		0.85±0.06		<0.01

**Comparing pre-test NO_x^- level and maximal ST depression at ET_1 and ET_2. **Significantly higher ($P<0.03$) versus pre-ET_2 value. ET Exercise test; ET_1 and ET_2 First and second exercise tests, respectively*

DISCUSSION

Our study shows that the plasma NO_x^- level increases slightly during ET_1 and significantly during ET_2, and there is a significant decrease of ST depression during ET_2, with peak MVO_2 remaining unchanged between the two tests. The attenuation of ECG ischemia during a similar ischemic stress is probably an expression of ischemic preconditioning. Because the protection was observed 24 h after the preconditioning episode, it was attributed to delayed ischemic preconditioning, considering that early preconditioning operates only in the first 2 h after the preconditioning episode (12,13). The observed pattern of evolution of plasma NO_x^- level is compatible with the theory that NO is a mediator of delayed ischemic preconditioning, alone or together with other mechanisms.

The maximal workload and peak DP during the two consecutive ETs at 24 h were very close. This suggests that training effect or changes of collateral circulation do not influence the parameters reached during ET_2 (14).

The contribution of early preconditioning to decreasing maximal ST depression during consecutive exercise testing at 30-min intervals was proved by Tomai et al (15,16) and confirmed by Zdrenghea et al (17). However, studies of delayed preconditioning during consecutive exercise stress testing yielded conflicting results. Two previous studies (4,18) revealed less ST depression during a second ET at 24-h intervals, as in the present study. However, Tomai et al (16) found no delayed protective effect of exercise-induced ischemia.

It was demonstrated experimentally in animals that the preconditioning ischemic episode increases NO production (by eNOS) and other mediators (adenosine, reactive oxygen species) which act synergistically as triggers for the iNOS transcription; the level of the enzyme increases after 24 h and lasts for 72 h (7,8). During this interval of time, a new ischemic episode will result in increased NO production with a decrease of the consequences of myocardial ischemia, the mechanisms of which are only partially known (19,20). However, delayed ischemic preconditioning appears to be a heterogeneous phenomenon, involving multiple inter-related effectors, both NO-dependent and NO-independent (20).

In a recent study (21), nitroglycerin (a NO donor) protected myocardium against PTCA-induced ischemia between 24 h to 72 h after its discontinuation. This supports the hypothesis that NO is not only an effector (as in our study) but also a trigger of delayed ischemic preconditioning.

All clinical studies on preconditioning, including ours, have some inherent limitations because of ethical reasons. Consequently, data are indirect and do not reveal the intimate mechanisms of changes observed.

Given these limitations, we cannot exclude subtle changes of myocardial perfusion from one ET to another, although maximum MVO_2 remained relatively unchanged.

In addition, we cannot identify the source of NO by systemic plasma NO_x^- quantitation. There is evidence that NO can be increased by shear stress, through constitutive NO synthases (22,23), in different cells. These sources of NO could be stimulated by increased perfusion on exercise. However, this does not explain the increase of NO_x^- only at ET_2.

Another problem is that beneficial effect of NO could be exerted not only by mechanisms of preconditioning, but also by 'classical' vasodilation, by direct reduction of contractility (24) or by diminishing the norepinephrine release (25).

Finally, the links between the evolution of plasma NO_x^- and ST changes and between ST changes and ischemic preconditioning are based only on circumstantial evidence, so it is difficult to make a causal relation between them relying solely on these data.

In summary, our study shows an increase of the plasma NO metabolites level after the second of two consecutive exercise stress tests at 24-h intervals, along with a decrease of electrocardiographic consequences of a similar ischemic stress. These findings could be related to delayed ischemic preconditioning, but caution is needed for interpretation.

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